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An investigation on hepatoprotective activity of entire plant of *Ipomoea pestigridis* (family Convolvulaceae) on hepatotoxicity induced rats

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ABSTRACT

The hepatoprotective potential of *Ipomoea pestigridis* (Family Convolvulaceae) on hepatotoxicity induced rats were investigated in the present study. *Ipomoea pestigridis* (Linn) (family Convolvulaceae) is "Tiger Foot Morning Glory" in English. Hepatotoxicity was induced by Paracetamol (2g/kg b.wt.) given to rats on the 5^{th} day of the investigational period and given orally. All three extracts(PE, EA and methanol) were administered to normal and experimental hepatotoxicity rats for 7 days. Paracetamol-induced hepatotoxicity and compared with Silymarin, a standard hepatoprotective reference drug. Liver marker enzymes (ALT, ALP, AST and GGT) and Serum (Total Bilirubin, Total Protein, Total Cholesterol, Triglycerides, Albumin, Urea and Creatinine) were evaluated. Paracetamol-induced rats to exhibit elevated activities of liver enzymes such as SGOT, SGPT, ALP, gamma-glutamyl transpeptidase (GGT), creatinine, TB, urea, TC, TG and reduced total protein serum. Furthermore, Oral administration of the EA concentrates of *Ipomoea pestigridis* (200 mg/ kgb.wt.) given rats were major reduction the level of SGOT, SGPT, ALP,gamma-glutamyltranspeptidase (GGT) creatinine, urea, TB, TC&TG and also significantly elevated the concentration of TB and albumin when compared to other concentrates. Thus, results suggested that EAconcentrates of *Ipomoea pestigridis* could afford better hepatoprotective activity against paracetamol treated hepatotoxicity rats.

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INTRODUCTION

The liver is a vital organ in the body as it provides defense from potentially injurious exogenous and endogenous compounds and in this method, it gets affected (Ghosh *et al.*, 2013). Liver illness is a universal problem. Conventional medicine utilized in the management of liver diseases are rarely insufficient and must be severe side effects. Thus, essential to investigate for substitute drugs for the management of liver disease in order to replace presently use the medicine of uncertain efficacy and safety (Özbek *et al.*, 2003).

Paracetamol acts as an antipyretic and analgesic agent used in the clinical field. During beneficial doses, paracetamol is metabolized by glucuronidation or sulfation by the cytochrome p450 structure into the reactive metabolite NAPQI. Under normal conditions, NAPQI is rapidly changed to harmless GSH. While a higher amount of paracetamol, ele-

vated concentration of NAPQ1 reacts with hepatic proteins and leads to liver injure (Coles *et al.*, 1988). Alternative medicines used for the management of liver diseases having adverse side effects and are costlier. Therefore, there is a requirement to assess natural molecules as an effective medicine that is safer and less costly.

Ipomoea pestigridis (Linn) (family Convolvulaceae) is generally known as "Tiger Foot Morning Glory" (Sahu and Gupta, 2014; Pawar and Patil, 2004) .*I.pestigridis* was used for management of wound healing (Austin, 1975; Amor-Prats and Harborne, 1993).

*I.pestigridis*was used for different diseases like headaches, swellings, poisonous stings, snake bites. *I.pestigridis*was used for analgesic, antimicrobial, thrombocytic, cytotoxic activity (Pratap *et al.*, 2011). Still, no literature is available on the hepatoprotective activity of the entire plant of *I.pestigridis*. Thus, the study to assess hepatoprotective activities of *Ipomoea pestigridis* (Roxb.) in hepatotoxicity rats.

MATERIALS AND METHODS

Gathering & Identification of Plant

The entire plant of *I.pestigridis* (family Convolvulaceae) was gathered form kalakkad, Tirunelveli District of Tamilnadu India. Plant identification was made from Botanical investigation of India, Palayamkottai The *I.pestigridis* were desiccated under shadowy, segregate, crushed through grinder (Satheeshkumar *et al.*, 2011).

Preparation of Concentrates

The pulverized materials were progressively concentrated with PE (40-60^{0}C) through hot constant percolation method in Soxhlet equipment (Harborne, 1984) for twenty-four hours. At that moment, the marc was used to EA (76-78^{0}C) for twenty-four hours & then mark was subjected to methanol for twenty-four hours (Shajiselvin *et al.*, 2010). The concentrates were concentrated through the rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired (Satheesh Kumar D, Kottai Muthu A and Manavalan R, 2010; Alagumanivasagam *et al.*, 2012).

Animals

Male Wister rats of 16-18 weeks age, weighing 150-160g, were collected from the Pharmacology department, MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana, India. Rats were set aside in cages, 2 per cage, with twelve: twelve hours light and dark cycle at 25 $\pm$ 2ºC. Rats were maintained on their particular diets and water *ad libitum*. Animal Ethical Committee's clearance was approved by the Ethical Committee of MNR College of Pharmacy, Sangareddy, Hyderabad, Telangana (CPCSEA/COP/10/16-09-2019).

Experimental design

Acute toxicity test

Albino Wistar rats were separated into six groups and each group contains six animals (n = 6). Rats have fasted for four hours with free access to water only. The various concentrates of *Ipomoea pestigridis* suspended in 0.5% CMC was administered orally at a dose of 5 mg/kg at first and mortality was noted for three days. The acute toxicity study was carried out OECD guideline 423.

Evaluation of Hepato protective activity

Animals were separated into six groups and each group contains six animals.

Group I: Animals served as Control group received with vehicle (0.5% CMC) for 7 days.

Group II: Animals served as a negative control, received 7 days only 1ml vehicle and paracetamol 2g/kg b.wt. given on the 5^{th}-day by orally

Group III: Animals received Pet. Ether concentrates on *Ipomoea pestigridis* 200mg/kg b.wt, orally for seven days. On 5^{th} day onwards paracetamol 2g/kg b.wt.administered by orally.

Group IV: Animals received ethyl acetate concentrates of *Ipomoea pestigridis* 200mg/kg b.wt, orally for seven days. On 5^{th} day onwards paracetamol 2g/kg b.wt.administered by orally.

Group V: Animals received a methanolic concentrate of *Ipomoea pestigridis* 200mg/kg b.wt, orally for seven days. On 5^{th} day on wards paracetamol 2g/kg b.wt.administered by orally.

Group VI: Animals received 25mg/kg b.wt of Silymarin by oral for seven days and on 5^{th} day onwards paracetamol 2g/kg b.wt.administered by orally (Sivakrishnan S, Kottai Muthu A, 2014).

Groups III, IV and V rats were orally fed with the various concentrates of *Ipomoea pestigridis* (PE, EA and methanol) and Group VI rats were fed with silymarin. Both the *Ipomoea pestigridis* concentrates and silymarin were suspended in 0.5% CMC individually and fed to the particular rats through oral intubation.

Next day of experiments 8^{th} day, all the animals were sacrificed through cervical decapitation. Blood was collected in test tubes in dry condition and allowed to coagulate at ambient temperature for 30m. The serum was removed through centrifugation at 2000 rpm for 10m. The removed serum was utilized for

the examination of liver enzymes.

Liver marker enzymes

Reitman and Frankel (1957) method was used to determining the SGOT and SGPT, and (Kind and King, 1954) method was to determine ALP. (Lowry *et al.*, 1951) method were to determine the Total Protein (TP) levels, and (Patton and Crouch, 1977) method was used to determination of Urea.

Jaffe (1986) The method was used to determination of creatinine and Henry and Winkelman (1974) method was utilized for the estimation of TC. (Mallay and Evelyn, 1937) method was used for the determination of Total Bilirubin (TB) and Foster and Dunn (1973) method was used for the determination of TG.

Statistical Analysis

The statistical investigation was conducted by Analysis of variance and groups were compared through Duncan's Multiple Range Test (DMRT) using SPSS Software Package, version 10.0. Results were expressed as means ± Standard Error for 6 rats in each group. A value of $P \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Acute Toxicity

In the present study shown that a lethal dose of various concentrates of *Ipomoea pestigridis* was showed the safety of concentrates. Administration of various concentrates of *Ipomoea pestigridis* in rats did not change any autonomic or behavioral reaction.

There was no mortality of various concentrates of *Ipomoea pestigridis* was recorded at 2000mg/kg. Acute toxicity results revealed that the LD50 value of 2000mg/kg. Hence, the therapeutic dose was considered as $1/10^{th}$ (200mg/kgb.wt.)concentrates was taken for further investigation.

The activity of various concentrates of *Ipomoea pestigridis* on average liver weight changes in rats

The activity of various concentrates of entire plants of *Ipomoea pestigridis* on average liver weight changes in normal and paracetamol-induced hepatotoxic rats appeared in Table 1. The paracetamol-induced rat's group showed gained liver weight. The administration of EA concentrates of *Ipomoea pestigridis* treated group rats were significantly reduced the liver weight and the silymarin treated group restored liver weight.

P values, *P<0.05; **P<0.01; ns= not significant; compared to Paracetamol group. One way ANOVA followed by Dunnett's test. a→ Group II compared to Group I; b→ Group II compared to Group III, IV, V and VI.

Effect of various concentrates of *Ipomoea pestigridis* on liver enzymes and other parameters in paracetamol-induced hepatotoxicity in Wistar rats

The effect of various concentrates of entire plants of *Ipomoea pestigridis* on hepatic marker enzymes in the serum from normal and paracetamol-induced hepatotoxic rats was shown in Table 2. The paracetamol-induced rats were showed increment activities of SGPT (177.95±0.73), SGOT (190.80±1.55), ALP (269.92±1.42) and GGT (2.11±0.02). Numerous studies have established. Paracetamol-induced liver damage is considered and also used for the toxic agent of liver toxicity. (Remien *et al.*, 2014).

Liver damages produced by treated Paracetamol in larger doses after undergoing metabolised to produce a toxic compound, N-acetyl-p-benzoquinone imine (NAPQI) in the presence of CP-450 mono-oxygenase (Dahlin *et al.*, 1984). Administration of PE concentrates of *Ipomoea pestigridis* showed considerably reduced the enzymes SGPT(172.71±0.75), SGOT(180.33±1.16), ALP(260.43±1.64) and GGT(1.91±0.03) in group III rats. Administration of EA concentrates of *Ipomoea pestigridis* showed considerably reduced the enzymes SGPT(66.81±0.71), SGOT(111.04±0.90), ALP(197.80±0.73)and GGT(1.42±0.01) in group IV rats.

Administration of methanolic concentrates of *Ipomoea pestigridis* showed considerably reduced the enzymes SGPT(137.30±0.92), SGOT(163.40±0.91), ALP(238.24±0.82) and GGT(1.72±0.01) in group IV rats. The administration of EA concentrates of *Ipomoea pestigridis* attenuated the hepatic marker enzymes may be due to the encouragement of glucuronidation (Brunton *et al.*, 1992).

The effect of various concentrates of *Ipomoea pestigridis* on Creatinine, Urea and Total Bilirubin (TB) in the serum from normal and hepatotoxic rats were summarized in Table 3.

The hepatotoxic control group showed increased the level of creatinine as 79.36±1.03, urea as 20.54±0.10 and TB as 4.84±0.04. Increased levels of TB reflect the deepness of liver disease and elevated amino transferases and ALP have clear expression of cellular leakage and cell integrity is loss (Saraswat *et al.*, 1993). The paracetamol treated group showed increased the level of Creatinine, Urea. This effect may be urea and creatinine

Table 1: Effect of various concentrates of *Ipomoea pestigridis* on average liver weight changes in rats

Group	Final Liver Weight(g/100g)
Group I	4.20±0.02
Group II	$6.28\pm0.06^{a*}$
Group III	$5.97\pm0.04^{b**}$
Group IV	$4.50\pm0.04^{b**}$
Group V	$5.42\pm0.02^{b*}$
Group VI	$4.38\pm0.01^{b*}$

Data be articulated as mean ± SEM., n = six rats each group.

Table 2: Activity of various concentrates of *Ipomoea pestigridis* on serum enzymes SGPT, SGOT, ALP and GGT in Paracetamol induced hepatotoxicity on Wistar rats

Group	SGPT ($IU.L^{-1}$)	SGOT ($IU.L^{-1}$)	ALP ($IU.L^{-1}$)	GGT ($IU.L^{-1}$)
Group I	64.44±1.13	991.84±1.45	190.50±1.21	1.36±0.02
Group II	$177.95\pm0.73^{a**}$	$190.80\pm1.55^{a**}$	$269.92\pm1.42^{a**}$	$2.11\pm0.02^{a**}$
Group III	$172.71\pm0.75^{b**}$	$180.33\pm1.16^{b**}$	$260.43\pm1.64^{b**}$	$1.91\pm0.03^{b**}$
Group IV	$66.81\pm0.71^{b**}$	$111.04\pm0.90^{b**}$	$197.80\pm0.73^{b**}$	$1.42\pm0.01^{b*}$
Group V	$137.30\pm0.92^{b**}$	$163.40\pm0.91^{b*}$	$238.24\pm0.82^{b*}$	$1.72\pm0.01^{b*}$
Group VI	$67.26\pm0.43^{b**}$	$101.20\pm0.97^{b**}$	$195.40\pm0.67^{b**}$	$1.38\pm0.02^{b**}$

Statistical informationand particulars of group I-VIwassimilar as in Table 1

derangements are possible in the presence of a large dose of paracetamol (Kale *et al.*, 2012).

Treatment of EA concentrates of *Ipomoea pestigridis* showed a reduced level of creatinine as 59.75±1.16, urea as 8.81±0.05 and TB as 1.77±0.01. Treatment of EA concentrates on *Ipomoea pestigridis* had a significant reduction in the creatinine, Urea and Total Bilirubin and silymarin restored Creatinine, Urea and Total Bilirubin to normal values. This effect may be due to the presence of flavonoids in the *Ipomoea pestigridis.*

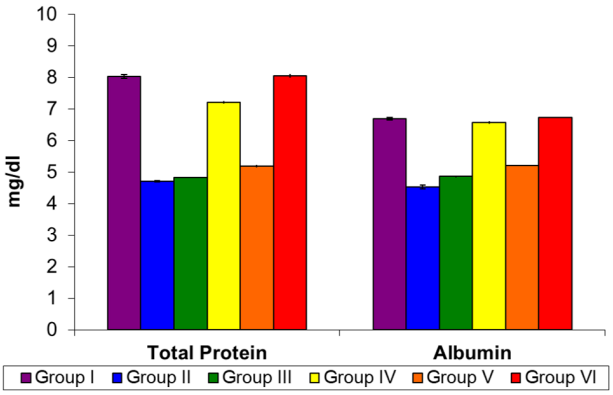

Figure 1: Activity of various concentrates of *Ipomoea pestigridis* on serum (TP and ALB) in paracetamol-induced hepatotoxicity on Wistar rats

The effect of various concentrates of *Ipomoea pestigridis* on TG and TC in the serum from normal and hepatotoxic rats were shown in Table 4. The hepatotoxic control group showed increased the level of TG as 89.69±0.94 and TC as 193.50±1.14that of a control group of rats. Triglyceride and total cholesterol concentrations were elevated in treated with Paracetamol-induced rats as compared to control rats (group I).

The liver is mainly ruling TC concentration in the body does not produce TC for goes to another cell but it also separates cholesterol from the body by changing to bile salts and eliminate to bile. Oral administration of PE concentrates showed reduced the level of TG and TC in group III rats as 80.30±1.15 and 189.87±0.50. Oral administration of EA concentrates showed reduced the level of TG and TC in group IV rats as 59.26±0.54 and 106.31±0.59.

The administration of EA concentrates on *Ipomoea pestigridis and* the silymarin treated group of significant rat reduction in the level of TG and TC as compared to PE and methanol concentrates. This effect could be due to diminished lipoprotein activity (You *et al.*, 2002).

The various concentrates of *Ipomoea pestigridis* on TP and albumin in the serum from normal and paracetamol-induced hepatotoxic rats were

Table 3: Activity of various concentrates of *Ipomoea pestigridis* on Creatinine, Urea and Total Bilirubin in Paracetamol induced hepatotoxicity on Wistar rats.

Group	Creatinine (mM^{-1})	Urea (mM^{-1})	Total Bilirubin (mg/dL)
Group I	56.52±0.86	7.82±0.04	1.55±0.02
Group II	$79.36\pm1.03^{a**}$	$20.54\pm0.10^{a**}$	$4.84\pm0.04^{a**}$
Group III	$77.11\pm1.34^{b**}$	$19.20\pm0.56^{b**}$	$4.60\pm0.03^{b*}$
Group IV	$59.75\pm1.16^{b**}$	$8.81\pm0.05^{b**}$	$1.77\pm0.01^{b**}$
Group V	$69.26\pm0.87^{b*}$	$14.98\pm0.09^{b*}$	$3.80\pm0.04^{b*}$
Group VI	$56.67\pm1.24^{b**}$	$8.16\pm0.04^{b**}$	$1.65\pm0.02^{b**}$

Statistical information and particulars of group I-VI was similar as in Table 1

Table 4: Activity of various concentrates of Ipomoea pestigridis on TG and TC in paracetamol-induced hepatotoxicity on Wistar rats

Group	TG (mg/dl)	TC (mg/dl)
Group I	57.35±0.59	96.97±0.85
Group II	$89.69\pm0.94^{a**}$	$193.50\pm1.14^{a**}$
Group III	$80.30\pm1.15^{b**}$	$189.87\pm0.50^{b**}$
Group IV	$59.26\pm0.54^{b**}$	$106.31\pm0.59^{b**}$
Group V	$69.38\pm0.66^{b*}$	$163.72\pm0.76^{b*}$
Group VI	$59.75\pm0.84^{b**}$	$100.79\pm0.79^{b**}$

Statistical information and particulars of group I-VI was similar as in Table 1

Table 5: Activity of various concentrates of *Ipomoea pestigridis* on serum (TP and ALB) in paracetamol-induced hepatotoxicity on Wistar rats

Group	TP (mg/dl)	ALB (g/dl)
Group I	8.02±0.06	6.68±0.04
Group II	$4.70\pm0.02^{a**}$	$4.53\pm0.06^{a*}$
Group III	$4.83\pm0.02^{b**}$	$4.86\pm0.03^{b*}$
Group IV	$7.21\pm0.04^{b*}$	$6.56\pm0.04^{b**}$
Group V	$5.18\pm0.04^{b**}$	$5.20\pm0.02^{b**}$
Group VI	$8.04\pm0.06^{b**}$	$6.72\pm0.02^{b**}$

Statistical informationand particulars of group I-VI was similar as in Table 1

depicted in Table 5 and Figure 1.

The hepatotoxic control group showed a reduction of TP as 4.70±0.02 and albumin as 4.53±0.06. Oral administration of PE concentrates showed considerable TP and ALB levels in group III rats as 4.83±0.02 and 4.86±0.03. Oral administration of ethyl acetate concentrates showed a marked reduction in TP and ALB levels in group IV rats as 7.21±0.04 and 6.56±0.04.

The administration of EA concentrates of *Ipomoea pestigridis* and silymarin treated rats were significantly elevated the level of TP and ALB as compared to PE and methanol concentrates. The effective elevation of TP and ALB can be attributed to an improvement in the hepatic cell's secretory mechanisms.

CONCLUSION

It is thus concluded that EAconcentrates of *Ipomoea pestigridis* has promising hepatoprotective activity, which potentially improved abnormalities of hepatotoxicity conditions in paracetamol-induced hepatotoxicity rats. The probable hepatoprotective effect may be due to the presence of phytoconstituents in the EA concentrates of *Ipomoea pestigridis.* However, further studies are in progress to isolate the active constituents of the EA concentrates of *Ipomoea pestigridis.*

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